

Reverse transcription

Performance meets stability

SuperScript V Reverse Transcriptase enables room-temperature setup, extended benchtop handling, and automation-friendly workflows

Introduction

Reverse transcription is a critical step in several RNA analysis workflows, such as gene expression analysis, pathogen detection, and molecular assay development. In conventional workflows, reverse transcription reagents and assembled reactions are typically handled under strict time and temperature conditions to help preserve enzyme activity and assay performance. These practices—including rapid thawing, cold storage during reaction setup, and prompt transfer to incubation—are well established but can limit workflow flexibility, particularly in higher-throughput and decentralized testing settings. Automated liquid-handling systems, staggered sample processing, multiple operators, and batch-based reaction setup all benefit from reagents that remain stable under ambient laboratory conditions. Room-temperature stability of reagents and reactions can simplify workflow design, reduce handling complexity, and improve operational efficiency without compromising assay performance.

This application note evaluates the room-temperature stability and workflow flexibility of Invitrogen™ SuperScript™ V Reverse Transcriptase and SuperScript™ V VILO™ Master Mix. Performance was assessed across conditions representative of routine laboratory use, including prolonged room-temperature reagent exposure, room-temperature reaction setup, and benchtop storage of assembled reactions. Enzyme thermostability relative to other commercially available reverse transcription reagents and previous-generation SuperScript products were also assessed. The results demonstrate that SuperScript V products retain robust reverse transcription performance under flexible handling conditions, enabling reliable room-temperature workflows for both manual and automated RNA analysis.

Benefits of reagent stability

The enhanced stability of SuperScript V Reverse Transcriptase and SuperScript V VILO Master Mix offers multiple workflow benefits (Table 1). The stability characteristics of these enzymes are particularly valuable for high-throughput and automated workflows, clinical and translational research laboratories, decentralized testing settings, biobanking, and applications involving challenging RNA templates.

Table 1. Workflow benefits enabled by the stability of SuperScript V products.

Stability attribute	Workflow benefit
Room-temperature reagent stability for up to 6 months*	Supports ambient shipping, simplifies routine reagent handling, and reduces reliance on continuous cold storage
Room-temperature setup and benchtop stability of assembled reactions for up to 24 hr	Enables room-temperature reaction setup, master mix staging, delayed incubation, and flexible batch processing
Reverse transcriptase thermostability for up to 65°C	Supports higher reverse transcription temperatures, improving access to structured, GC-rich, or long-RNA templates.

* 6 months for SuperScript V Reverse Transcriptase; 1 month for SuperScript V VILO Master Mix.

Materials and methods

Ambient shipping simulation

To mimic routine transportation and laboratory receipt, SuperScript V reagents were subjected to simulated ambient shipping conditions consisting of 2 hours at -20°C , 2 hours at 40°C , and ≥ 48 hours at 25°C . These cycling conditions were repeated 3 times. All other room-temperature stability studies presented in this application note were performed using reagents following this simulated shipping procedure.

Room-temperature stability of SuperScript V VILO Master Mix

Aliquots of SuperScript V VILO Master Mix were stored at room temperature for 1, 3, 7, 10, 14, 30, or 60 days. Frozen aliquots stored at -20°C served as controls. Reverse transcription was performed using 10 ng HeLa total RNA in 20 μL reactions. cDNA was analyzed by qPCR using Applied Biosystems™ TaqMan™ Fast Advanced Master Mix and TaqMan™ Gene Expression Assays targeting high- (*GAPDH*), medium- (*NUF*), and low-abundance (*REN*) targets. To assess quantitative performance (linearity) across a broad dynamic range, synthetic RNA control transcript within the External RNA Controls Consortium reference set (ERCC-00150 RNA, 10^3 – 10^{11} copies) was reverse-transcribed in the presence of 100 ng HeLa total RNA. Master mixes stored for 60 days at room temperature or -20°C were evaluated. Efficiency and correlation coefficient (R^2) were determined in qPCR using TaqMan Fast Advanced Master Mix.

SuperScript V Reverse Transcriptase room-temperature stability

SuperScript V Reverse Transcriptase (200 U/ μL) was stored at room temperature for up to 6 months. Enzyme activity was measured and compared with the control enzyme stored continuously at -20°C . Activity of the control sample was defined as 100%, and the activity of room temperature–stored samples was expressed relative to this control. One unit of SuperScript V Reverse Transcriptase is defined as the amount of enzyme required to incorporate 1 nmol of deoxyribonucleotide into acid-precipitable material in 10 minutes at 37°C .

Room-temperature stability of ezDNase Master Mix

Aliquots of Invitrogen™ ezDNase™ Master Mix were stored at room temperature for up to 60 days and compared with frozen controls. Genomic DNA removal reactions were performed following the user guide using 50 ng human genomic DNA in the presence of 1 μg HeLa total RNA. Residual genomic DNA was quantified by qPCR using TaqMan Fast Advanced Master Mix targeting *GAPDH*, and DNA removal efficiency was calculated relative to untreated controls.

Room-temperature reaction setup

Reverse transcription reactions were assembled either at room temperature or on a cooling rack and remained under the respective setup conditions for up to 45 minutes before reverse transcription. Reactions contained either 10 ng or 100 pg HeLa total RNA. Twenty-four independent reactions were prepared for each condition. RT-qPCR was performed using TaqMan Fast Advanced Master Mix targeting *MYC*, and assay reproducibility was evaluated using C_t values and coefficients of variation (CVs).

Benchtop stability of assembled reverse transcription reactions

Reverse transcription reactions were set up using SuperScript V VILO Master Mix, with a series of dilutions of HeLa total RNA as input. Upon assembly, one set of reactions was immediately transferred to a thermal cycler for reverse transcription. The other set of reactions was left at room temperature ($\sim 25^{\circ}\text{C}$) for 4–24 hours before running reverse transcription reactions. cDNA samples were analyzed by qPCR using either Applied Biosystems™ PowerTrack™ SYBR™ Green Master Mix or TaqMan Fast Advanced Master Mix targeting *GAPDH*, or endpoint PCR using Invitrogen™ Platinum™ SuperFi™ II Green PCR Master Mix targeting *ACTB* (433 bp).

Reverse transcriptase thermostability

cDNA was synthesized from Invitrogen™ Millennium™ RNA Markers (0.5–9 kb) using SuperScript V Reverse Transcriptase and previous-generation Invitrogen™ SuperScript™ reverse transcriptases across a temperature range of 37 – 65°C . Results were analyzed by electrophoresis. In a separate experiment, reverse transcription reactions were performed using 10 ng HeLa total RNA while varying incubation temperature from 45°C to 70°C . Results were analyzed by qPCR using TaqMan Fast Advanced Master Mix targeting *MYC*.

Results

Room-temperature stability supports ambient shipping and routine laboratory handling

Reverse transcription reagents are typically transported and handled under refrigerated or frozen conditions. Greater tolerance to ambient conditions can simplify logistics, reduce sensitivity to temporary cold-chain interruptions, and offer additional flexibility during laboratory receipt and routine use. Following simulated ambient shipment, SuperScript V reagents were stored at room temperature and evaluated for RT-qPCR performance (Figures 1 and 2) and enzyme activity (Figure 3).

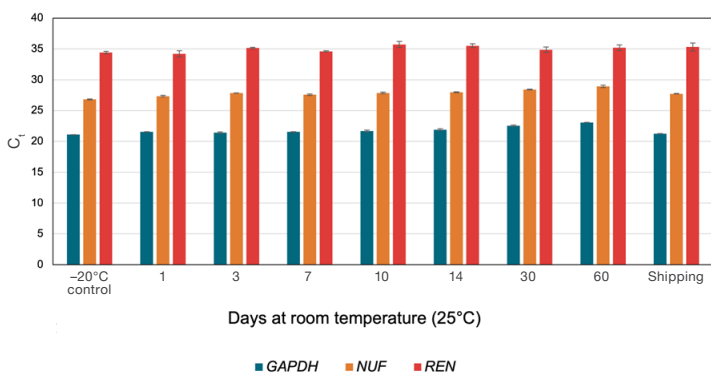


Figure 1. Consistent RT-qPCR performance with SuperScript V VILO Master Mix even after prolonged storage at room temperature. The stability of SuperScript V VILO Master Mix was evaluated by RT-qPCR, by comparing C_t values of the control master mix with the master mix incubated at room temperature for up to 60 days and the master mix after a shipping simulation at room temperature.

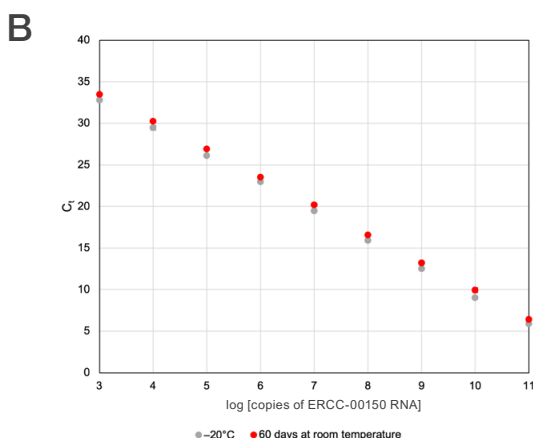
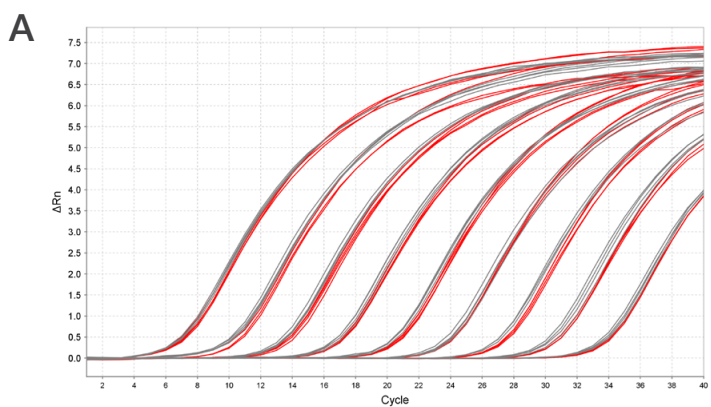


Figure 2. Excellent linearity with SuperScript V VILO Master Mix after storage at room temperature for 60 days. Stability of SuperScript V VILO Master Mix was evaluated by RT-qPCR by comparing control master mix stored at -20°C with master mix incubated at room temperature for 60 days. (A) Amplification plots and (B) standard curves demonstrating linearity across a broad dynamic range. Efficiency was 98% for the -20°C control and 97% for the room temperature incubation; R^2 was 1 for both the -20°C control and room temperature incubation.

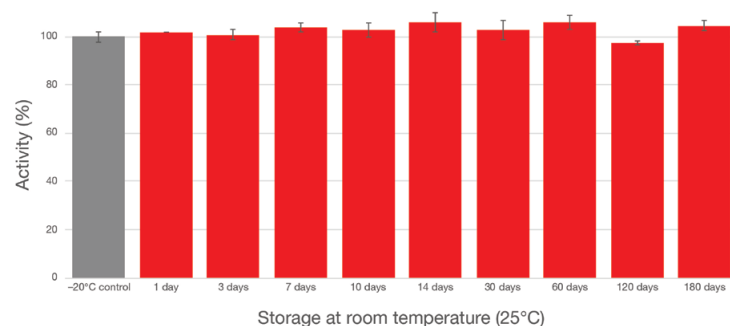


Figure 3. Change in enzyme activity over extended storage at room temperature. SuperScript V Reverse Transcriptase retains activity for 6 months.

SuperScript V VILO Master Mix includes Invitrogen™ ezDNase™ Master Mix for fast and simple reduction of genomic DNA. To further assess room temperature stability, the accompanying ezDNase Master Mix was also evaluated (Figure 4).

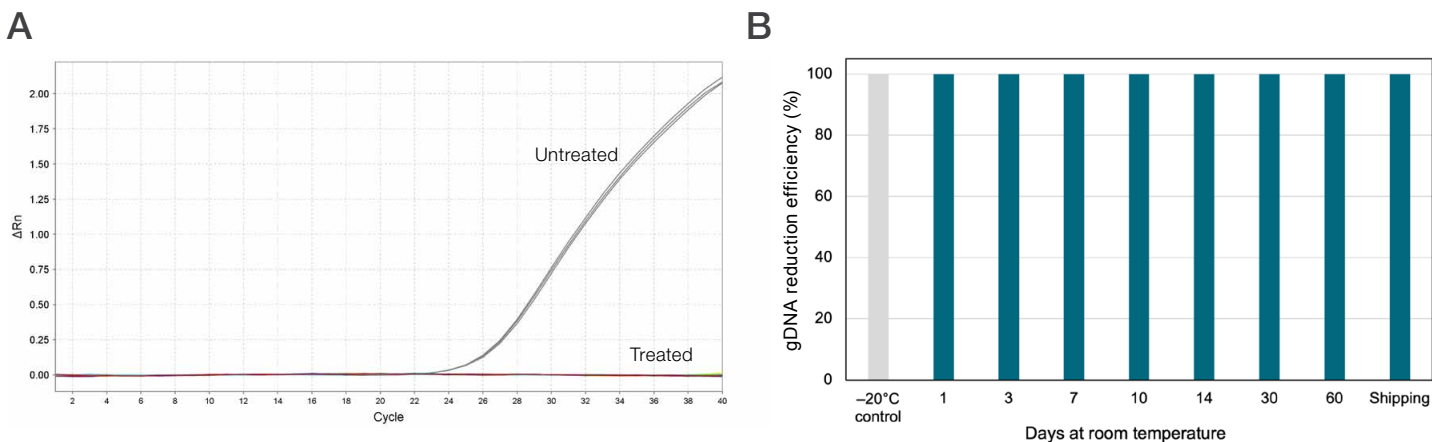


Figure 4. Efficient genomic DNA reduction with ezDNase Master Mix even after prolonged incubation at room temperature. Stability of ezDNase Master Mix was evaluated by RT-qPCR by comparing the control master mix with the master mix incubated at room temperature for up to 60 days, and the master mix after a shipping simulation at room temperature. **(A)** Amplification plots of samples treated with ezDNase Master Mix and untreated samples. **(B)** qPCR results are shown as gDNA reduction efficiency, calculated by comparing samples treated with ezDNase Master Mix versus untreated sample: $\text{gDNA reduction efficiency} = 100 - 100/2^{(C_t \text{ treated sample} - C_t \text{ untreated sample})}$.

Room-temperature setup and benchtop stability simplify reverse transcription workflows

Room-temperature reaction assembly can reduce dependence on ice, cooling racks, and cooled instrument decks (Figure 5). Stability of assembled reactions can also offer greater flexibility for staging, plate queueing, and delayed incubation. Room-temperature reaction setup produced RT-qPCR performance comparable to setup on a cooling rack, with minimal differences in C_t values and high reproducibility.

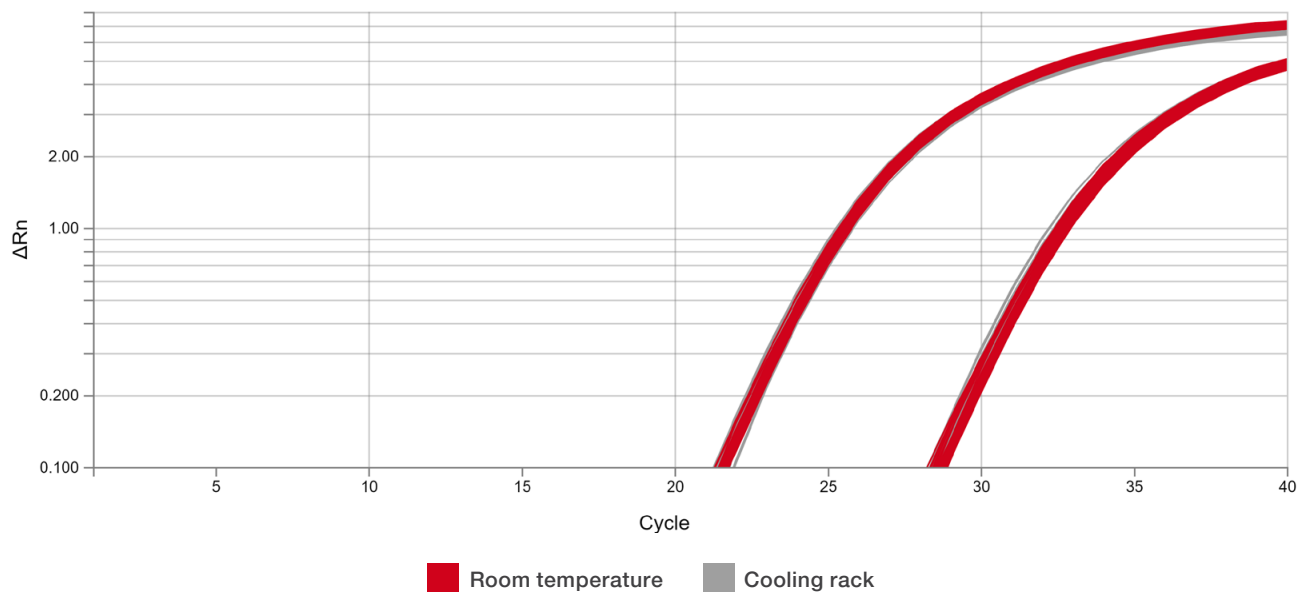


Figure 5. Reaction setup at room temperature vs. on a cooling rack (automation compatibility). SuperScript V VILO Master Mix reactions were assembled either at room temperature or on a cooling rack and incubated for up to 45 minutes during reaction setup. Subsequent RT-qPCR analysis showed comparable C_t values between setup conditions, with ΔC_t values below 0.3 cycles across both RNA inputs. High reproducibility was observed for all conditions (CV <0.8%), demonstrating that room-temperature reaction setup offers equivalent cDNA synthesis and downstream RT-qPCR performance without the need for a cooling rack.

In addition to supporting room-temperature assembly, SuperScript V VILO Master Mix was evaluated for stability after the reactions were fully assembled and held at room temperature (Figures 6 and 7). The results demonstrate that SuperScript V VILO Master Mix supports both room-temperature reaction assembly and prolonged benchtop holding. These capabilities enable master mix staging, delayed incubation, flexible plate queueing, and simplified manual and automated workflows without continuous cold handling.

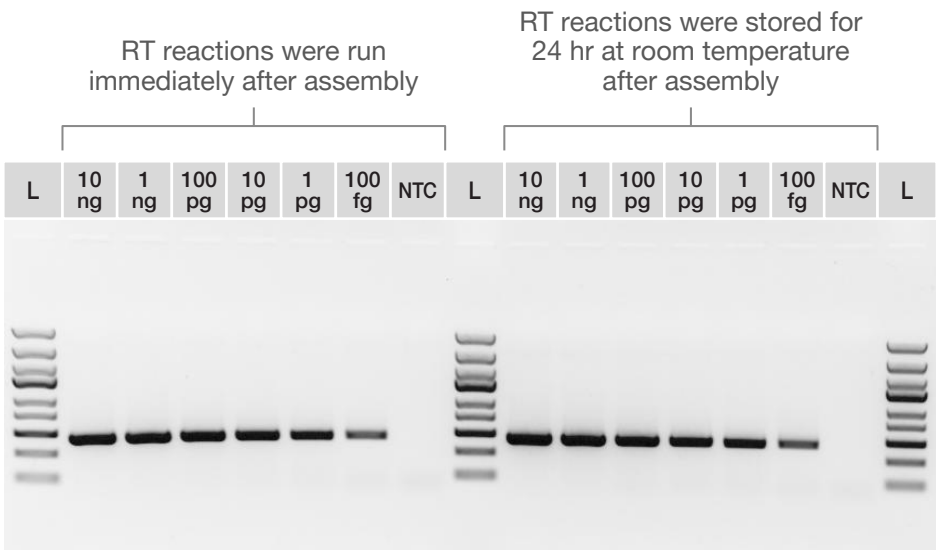


Figure 6. Assembled SuperScript V VILO Master Mix reactions remain stable for up to 24 hours at room temperature. RT-PCR product yield remained consistent across a broad RNA input range when reactions were processed immediately or held at approximately 22°C before reverse transcription. Ladder: Thermo Scientific™ GeneRuler™ Express DNA Ladder (Cat. No. SM1551); target: 433 bp (*ACTB*).

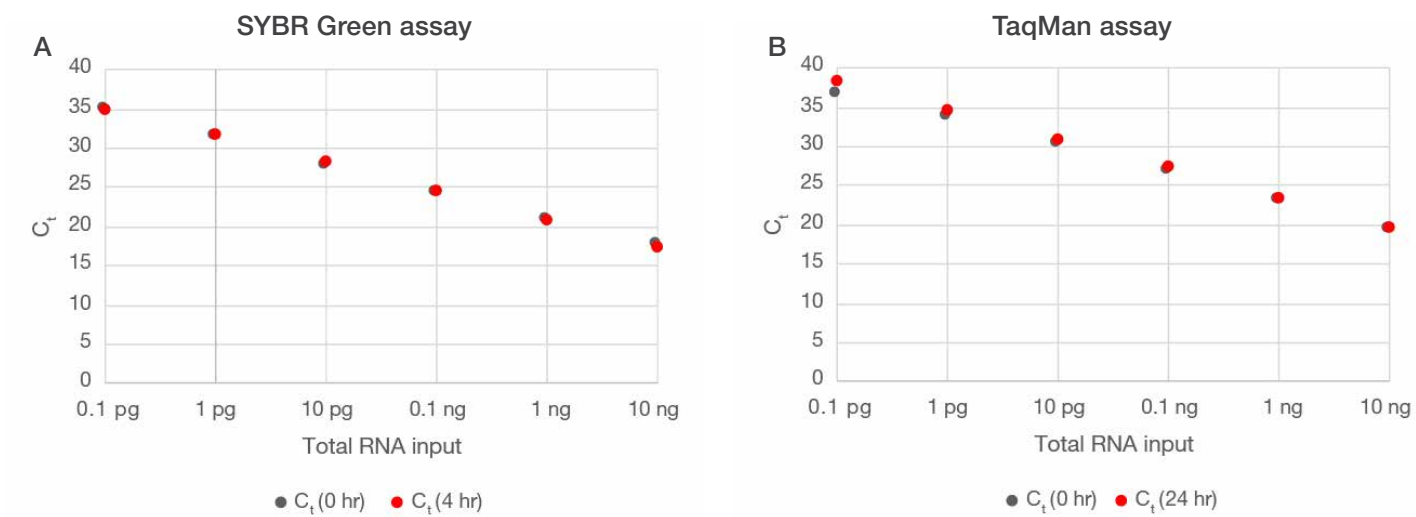


Figure 7. Maintenance of RT-qPCR sensitivity after assembled reactions are held for up to 24 hours at room temperature. C_t values remained consistent following up to 24-hour room-temperature holds. qPCR assays used either (A) Applied Biosystems™ SYBR™ Green dye or (B) TaqMan primers and probe for detection.

Reverse transcriptase thermostability supports challenging RNA templates

Elevated reverse transcription temperatures can help melt RNA secondary structure and improve access to structured or GC-rich regions. SuperScript V Reverse Transcriptase was evaluated across a broad temperature range using long-RNA and RT-qPCR assays (Figures 8 and 9). The results demonstrate that SuperScript V Reverse Transcriptase enables greater temperature flexibility for optimization of challenging RNA targets.

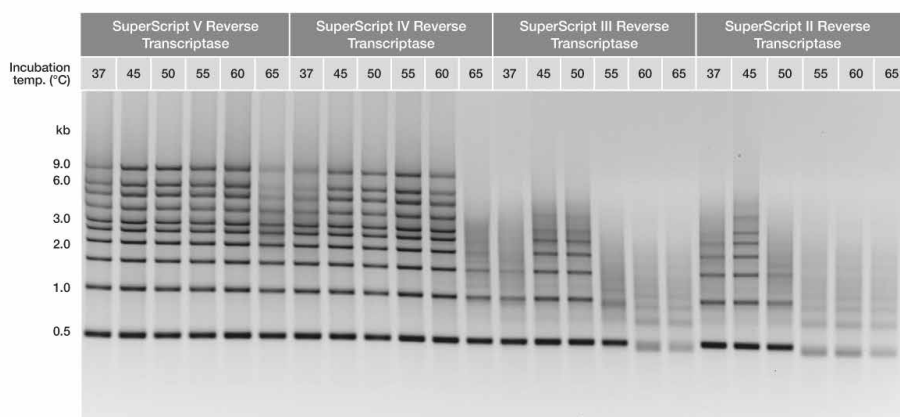


Figure 8. SuperScript V Reverse Transcriptase supports long-cDNA synthesis across a broad temperature range. cDNA products up to 9 kb were generated from Millennium RNA Markers at temperatures from 37°C to 65°C.

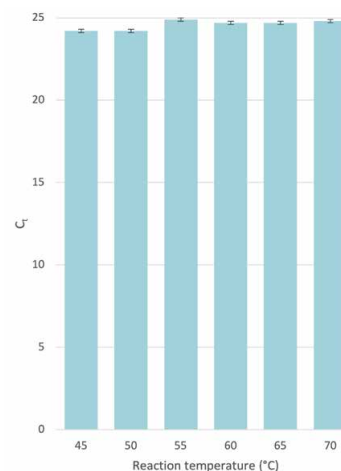


Figure 9. SuperScript V VILO Master Mix maintains RT-qPCR performance at elevated reverse transcription temperatures. C_q values remained consistent across reaction temperatures from 45°C to 70°C.

Conclusions

SuperScript V Reverse Transcriptase and SuperScript V VILO Master Mix maintained robust performance following simulated ambient shipment, prolonged room-temperature storage, room-temperature reaction setup, and extended benchtop holding of assembled reactions. Performance was preserved across experiments evaluating RT-qPCR, enzyme activity, and genomic DNA reduction.

SuperScript V products also supported long-cDNA synthesis and consistent reverse transcription across a broad temperature range. Together, these attributes simplify manual, automated, and high-throughput workflows by reducing cold-handling and timing constraints.

Learn more at thermofisher.com/superscript

invitrogen